Weighing brain activity with the balance: Angelo Mosso's original manuscripts come to light.

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Weighing brain activity with the balance: Angelo Mosso's original manuscripts come to light

Stefano Sandrone,[1,2,3] Marco Bacigaluppi,[1,2,4] Marco R. Galloni,[5] Stefano F. Cappa,[1,6]
Andrea Moro,[7] Marco Catani,[8] Massimo Filippi,[1,4,9] Martin M. Monti,[10] Daniela Perani[1,6,11,*]
and Gianvito Martino[1,2,*]

1 Vita Salute San Raffaele University, I-20132 Milan, Italy
2 Neuroimmunology Unit, Institute of Experimental Neurology, Division of Neuroscience, IRCCS San Raffaele Hospital, I-20132 Milan, Italy
3 Institute for Advanced Study IUSS – Pavia, I-27100 Pavia, Italy
4 Department of Neurology, Institute of Experimental Neurology, IRCCS San Raffaele Hospital, I-20132 Milan, Italy
5 Department of Veterinary Morphophysiology and Scientific and Technological Archives, University of Torino, I-10095 Grugliasco, Turin, Italy
6 Centre of Excellence for High-Field Magnetic Resonance Imaging (CERMAC) and Division of Neuroscience, IRCCS San Raffaele Hospital, I-20132 Milan, Italy
7 Ne.T.S. – Centre for Neurolinguistics and Theoretical Syntax, Institute for Advanced Study IUSS – Pavia, I-27100 Pavia, Italy
8 NATBRAINLAB – Neuroanatomy and Tractography Brain Laboratory, Department of Forensic and Neurodevelopmental Sciences, Institute of Psychiatry, King's College, SE5 8AF London, UK
9 Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, IRCCS San Raffaele Hospital, I-20132 Milan, Italy
10 Department of Psychology, University of California Los Angeles, Los Angeles CA, 90095, USA
11 Department of Nuclear Medicine, Division of Neuroscience, IRCCS San Raffaele Hospital, I-20132 Milan, Italy

*These authors contributed equally to this work.

Correspondence to: Stefano Sandrone,
Neuroimmunology Unit,
Institute of Experimental Neurology (INSpe), Division
of Neuroscience,
San Gabriele Building-DIBIT 2 A4B4,
IRCCS San Raffaele Hospital,
Via Olgettina 58, I-
20132 Milano, Italy
E-mail: stefano.sandrone@kcl.ac.uk or martino.gianvito@hsr.it

Neuroimaging techniques, such as positron emission tomography and functional magnetic resonance imaging are essential tools for the analysis of organized neural systems in working and resting states, both in physiological and pathological conditions. They provide evidence of coupled metabolic and cerebral local blood flow changes that strictly depend upon cellular activity. In 1890, Charles Smart Roy and Charles Scott Sherrington suggested a link between brain circulation and metabolism. In the same year William James, in his introduction of the concept of brain blood flow variations during mental activities, briefly reported the studies of the Italian physiologist Angelo Mosso, a multifaceted researcher interested in the human circulatory system. James focused on Mosso's recordings of brain pulsations in patients with skull breaches, and in the process only briefly referred to another invention of Mosso's, the 'human circulation balance', which could non-invasively measure the redistribution of blood during emotional and intellectual activity. However, the details and precise workings of this instrument and the experiments Mosso performed with it have remained largely unknown. Having found Mosso's original manuscripts in the archives, we remind the scientific community of his experiments with the 'human circulation balance' and of his establishment of the conceptual basis of non-invasive functional neuroimaging techniques. Mosso unearthed and investigated several critical variables that are still relevant in modern neuroimaging such as the 'signal-to-noise ratio', the appropriate choice of the experimental paradigm and the need for the simultaneous recording of differing physiological parameters.

Introduction

Functional brain imaging techniques, such as positron emission tomography (PET) and functional magnetic resonance imaging (MRI), are now central to evaluating organized neural systems in task-driven and resting states, in both healthy and pathological conditions. Behind the scenes of modern neuroimaging is 'the quest for an understanding of the functional organization of the (...) human brain, using techniques to assess changes in brain circulation', a search that 'has occupied mankind for more than a century' (Raichle, 1998). We currently know that the actual physiological relationship between brain function and blood flow changes was first investigated in 1890 by Charles Smart Roy and Charles Scott Sherrington. Despite their promising studies, interest in this topic ceased until the end of the 1920s because of the lack of appropriate scientific devices and the great influence of Leonard Hill, Hunterian Professor at the Royal College of Surgeons in England, who stated that no relationship existed between cerebral function and cerebral circulation (Hill, 1896; Raichle, 2009), a statement that remained unchallenged until a clinical report by John Farquhar Fulton (Fulton, 1928). However, previous reference to changes in brain blood flow during mental activities can be found on page 97 of the first volume of Principles of Psychology (James, 1890). While introducing the concept of changes in brain blood flow during mental activities, James specifically mentions the investigations of Angelo Mosso (1846– 1910), 'the foremost Italian physiologist of his time and his generation' (Anonymous, 1946; Sandrone et al., 2012) (Fig. 1). In the late 1870s, i.e. 10 years before Roy and Sherrington's research, Mosso moulded his previous observations into the hypothesis that an attentional or cognitive task can locally increase cerebral blood flow. To test this idea experimentally, Mosso conceived the plethysmograph, a device that could measure cerebral blood flow variations by recording brain pulsations in patients with skull defects (Mosso, 1881; Cabeza and Kingstone, 2001). This invention established the so-called 'Mosso method', which was a valuable approach to measuring blood flow variations and quantifying the magnitude of the organ volume changes by converting brain pulsation into plethysmographic waves (Zago et al., 2009). Using this method, Mosso was able to measure the changes in cerebral blood volume that occurred subsequently to cognitive tasks, such as performing mathematical calculations in patients suffering from a wide frontal skull breach (Mosso, 1881; Berntson and Cacioppo, 2009; Zago et al., 2009). These observations led Mosso to conclude that alterations in blood flow to the brain were determined by functional changes (Raichle, 2009). The demonstration of a local increase in blood flow during mental activities
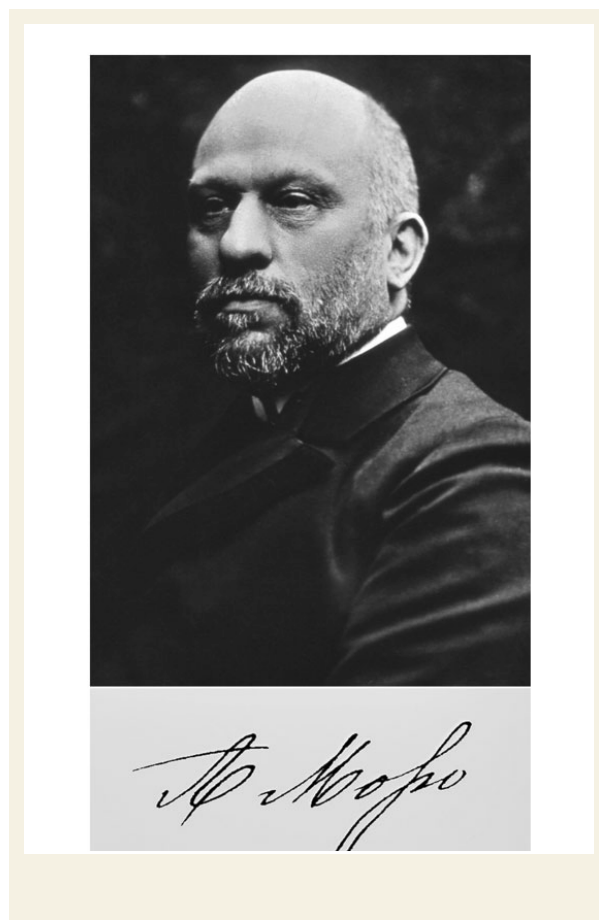
Figure 1 Photograph of Angelo Mosso and his signature (courtesy of Marco R. Galloni).

in patients with skull defects encouraged William James to enthusiastically affirm that this was 'the best proof of the immediate afflux of blood to the brain during mental activity' (James, 1890). Although extremely interesting, 'Mosso method' was only applicable to patients with skull breaches, and could not be used to assess brain flow variations in healthy subjects. To overcome this limitation, Mosso developed the 'human circulation balance' (Mosso, 1882), cited by James as a 'delicately balanced table which could tip downwards either at the head or the foot if the weight of either end were increased' (James, 1890). Notably, the crucial importance of the 'human circulation balance' was not entirely appreciated by James, who indeed refers mostly to the plethysmograph rather than to the balance when reporting Mosso's experiments, a bias that probably results from the fact that Mosso's works on the balance were written almost entirely in Italian. This language barrier may also explain why subsequent mention of the exact operating procedures of the balance were only rarely quoted (Lowe, 1936, but see also the citing of the balance by George Oliver during the 1896 Croonian Lecture), and Mosso's experiments with the balance have never, to the best of our knowledge, been previously reported in detail. Our current rediscovery of Mosso's work is the first to be based on his original writings (Mosso, 1882, 1884; Fig. 2 and Appendix 1) and indirect reports (Mosso, 1935) in the Archives of Turin, in Italy. Moreover, we put the 'human circulation balance' under the spotlight through the lens of contemporary neuroscience and discuss in detail its operating mechanism, the studies it performed, the experimental procedures and confounding variables, as well as the limitations and issues, that Mosso had to contend with.

How the balance works

The 'human circulation balance' invented by Mosso consisted of a wooden table lying on a fulcrum (Fig. 3). Subjects were first asked to lie down on the balance and not to move. Subsequently, after an initial adaptation phase needed for the blood to redistribute equally within the bodily tissues, the subject was steadily repositioned so as to overlap the barycentre with the central pivot of the fulcrum. This overlap was partially achieved by careful regulation of balance weights but also, as Mosso showed, by adjustments to the level of water inside a glass bottle positioned on one side of the table (Mosso, 1884). Once equilibrium was reached, the only observable movement was that induced by breathing during inspiration. Because this might cause a transitory increase in blood flow towards the lower extremities, the wooden table was linked to a heavy counterweight to dampen respiratory fluctuations. Mosso carefully detailed the procedure in order to allow anyone to build such an apparatus by themselves (Mosso, 1884; Fig. 4).

Interestingly, Mosso paid particular attention to building a machine that ensured the experimental subject would be comfortable; one instance of this attention was the padding Mosso placed on the table that he used for recording sessions (Mosso, 1884).
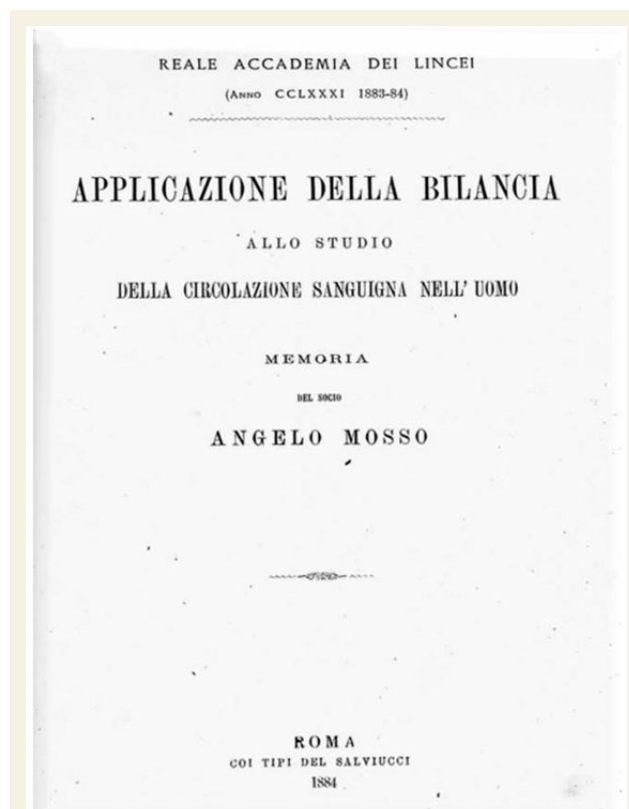

REALE ACCADEMIA DEI LINCEI
(Anno CCLXXXI 1883-84)

APPLICAZIONE DELLA BILANCIA

ALLO STUDIO

DELLA CIRCOLAZIONE SANGUIGNA NELL' UOMO

MEMORIA

DEL SOCIO

ANGELO MOSSO
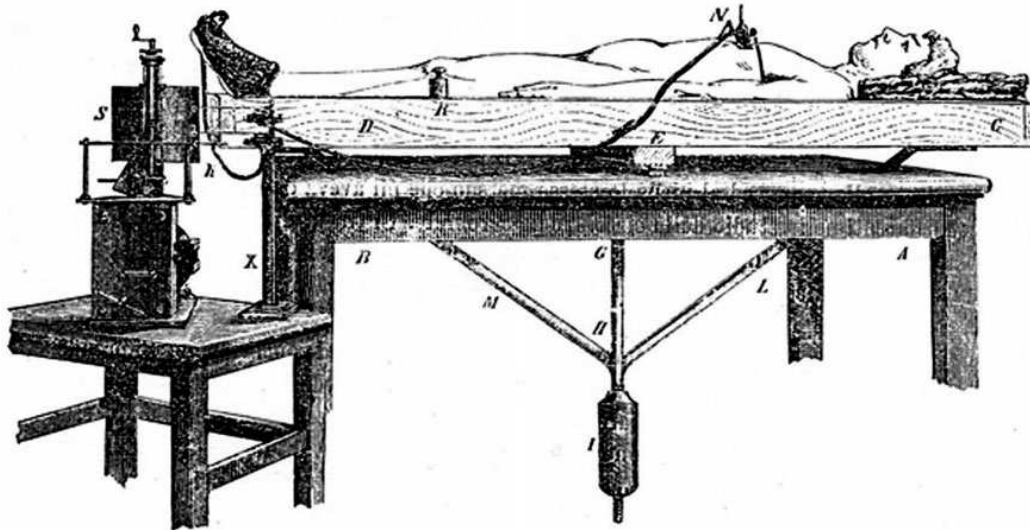
ROMA
COI TIPI DEL SALVIUCCI
1884

Figure 3 Mosso's 'human circulation balance', used to measure cerebral activity during resting and cognitive states. A and B = wooden table with three apertures on its top; C and D = tilting bed; E = pivot with steel knife fulcrum; G and H = 1 m long iron rod bearing the counterweight; I = cast iron counterweight with screw regulation; M and L = two iron stiffening bars; N = pneumatic pneumograph; R = equilibrating weight; S = kymograph; X = vertical stand for graphic transducers (Angelo Mosso's original drawing, modified and adapted from Mosso, 1884, Atti della Reale Accademia dei Lincei).
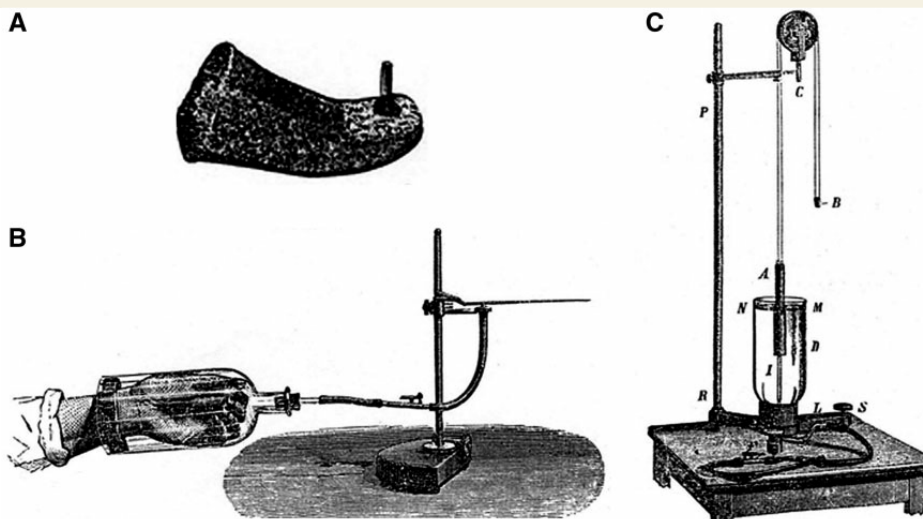
Figure 4 Angelo Mosso's original drawing of other components of the 'human circulation balance'. (A) Pneumatic sphygmograph made with gutta-percha and connected by a rubber tube with a Marey drum capable of transcribing the pulse of the foot. (B) Pneumatic sphygmograph for the hand, which is inserted into a glass bottle sealed around the wrist with soft cement. (C) Pneumatic plethysmograph. A = thin metal floating bell; B = counterweight with an ink pen writing on a kymograph; C = pulley; D = glass jar full of ether or petrol essence; F = rubber tube receiving air from a hand or foot blood pressure transducer; I = glass tube entering the jar from below and connected with tube F; L = tripod stand; N and M = level of liquid; R = vertical rod; S = levelling screw (modified and adapted from Mosso, 1884, Atti della Reale Accademia dei Lincei).

The subject's body was set in equilibrium as described above, with its respiration movements causing only slight, regular oscillation in the wooden horizontal table of the balance (Appendix 1).

Experimental variables and limitations of the balance

Several confounding variables needed to be reconciled for blood flow analysis to be valid, and Mosso was certainly aware of them. Indeed, he was determined to render the experimental conditions as 'close to normal' as possible (Mosso, 1884; Fig. 6 shows Mosso's laboratory in Turin). In particular, he struggled to identify equilibrium between the ecology of the set-up and the need to record the differing parameters required to understand the effect of each variable (Fig. 5A–D). Mosso accounted for head motion and other voluntary movements by setting reference points on the wooden table and using said points to identify the original position of the subject (Mosso, 1884). Physiological breathing-induced movements and those of the balance itself were recorded with a pneumatic pneumograph, an instrument that was invented by Jules Marey (1865) and modified by Mosso. A belt encircled with a flexible membrane on a metal drum was used to evaluate thorax movements, as breathing in and out caused variations in drum volume. These variations were then simultaneously registered on paper with a kymograph, an instrument invented by Carl Ludwig (1852). The kymograph consists of a drum that is covered by a paper sheet and rotated by a clockwork mechanism at different speeds so that an ink pen or a fine stick could draw a line depicting the variation in time of this physiological parameter.

Furthermore, Mosso also considered the concurrent changes in the volume of feet and hands to be a major variable during the recordings: these changes were co-registered with a hydraulic plethysmograph (Mosso, 1884; Figs 4, 6 and 7). Overall, despite Mosso's keen awareness of the number of artefacts that might arise from this procedure, together with his extensive efforts to quantify possible confounding variables, it is not clear whether the 'Mosso method' could realistically and sensibly discriminate between the signal (real brain blood flow changes) and the noise that, as Mosso himself stated, 'must be distinguished from other, psychically-induced types of blood movement' (Mosso, 1884) (Appendix 1).

The balance at work: Mosso's experiments

In 1884, Mosso reported the first results of the experiments per- formed on two healthy subjects, V.G, a 22-year-old medical student and Giorgio M., Mosso's laboratory technician. Mosso wrote that, to avoid artefacts, 'the participant initially spent at least one hour supine on the balance', and was sometimes overtly asked, during this so-called 'resting period', to relax (Mosso, 1884). With his balance, Mosso was able to measure blood flow variations in several organs, and in particular the pulmonary changes occurring during respiratory movement (Appendix 1). Mosso used the ba- lance not only to measure blood flow alterations as caused by respiratory movements, but also, towards the end of his career, to study the blood flow effects of emotional tasks. After the 'resting' period, Mosso presented the subjects with varying types of experimental conditions and measured any tilt in the balance towards the head-side.
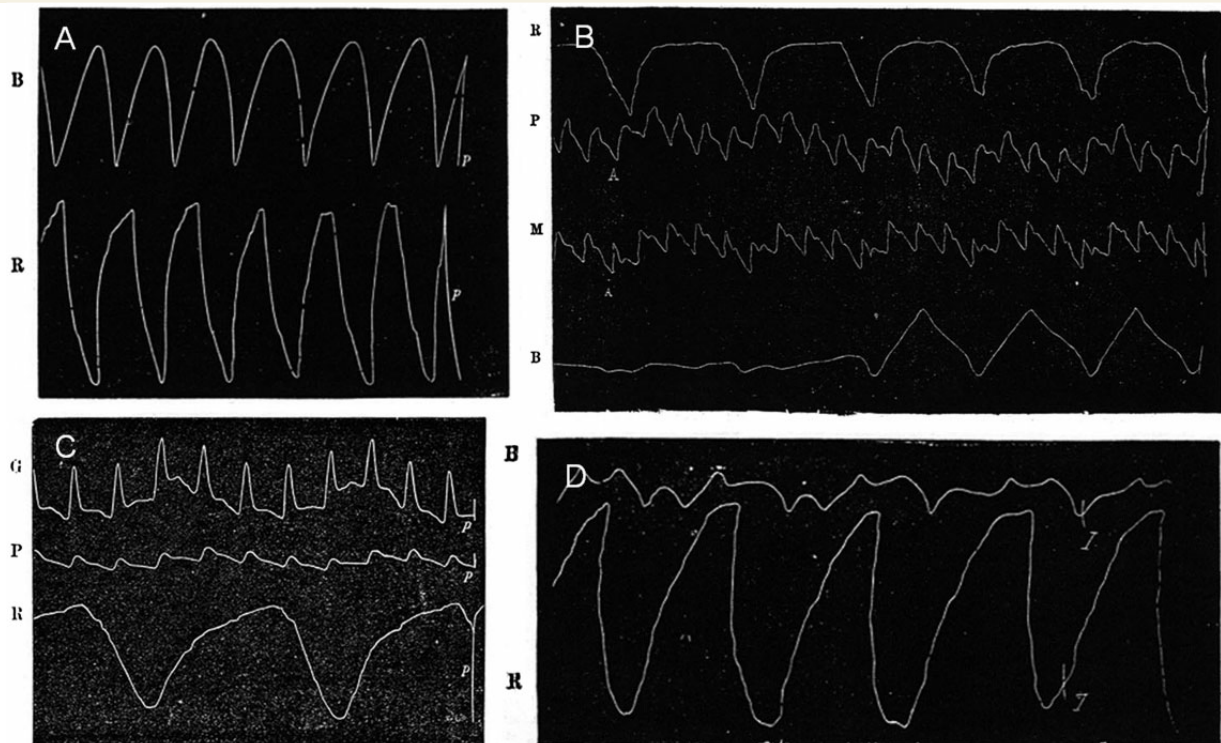
Figure 5 Angelo Mosso's original recordings. (A) Paper tracing of the balance movements (top line B) and of breathing (R). (B) Paper tracing of four parameters: R = breathing; P = foot pulse; M = hand pulse; B = balance movements. Foot and hand pulses are opposites (simultaneously time maximum in one and time minimum in the other). The left of the foot curve shows an initial accumulation of blood in the distal end, which causes the balance to remain in the lower position; a regular oscillation starts when the blood distributes more evenly through the body. (C) Paper tracing of three parameters: G = leg pulse; P = foot pulse; R = breathing. This experiment was intended to evaluate the separation in time of the maximum blood rush in the leg and in the foot; Mosso could see that the pulse takes ""2 s to cover the distance in the limb. (D) Paper tracing of the balance movements (top line B) and of breathing (R) with the subject sitting and the diaphragm muscle moving vertically on the axis of the pivot. Line B shows a flutter caused by a rubber dumper that was necessary to reduce wave amplitude (modified and adapted from Mosso, 1884, Atti della Reale Accademia dei Lincei).

Figure 6 Angelo Mosso's laboratory in Turin (courtesy of Marco R. Galloni).

Figure 7 Angelo Mosso performing one of his experiments. Here Mosso is photographed with his pneumograph at pneumatic transduction with two drums, an evolution of Marey's pneumograph, which in contrast had only one drum with a flexible guttapercha membrane (courtesy of Marco R. Galloni).

In his last experimental set-up (Appendix 1), Mosso's first stimulus was the sound of his hand hitting 'the knob of an electric key, just like those used to transmit telegrams' (Mosso, 1884), whereupon he observed that the balance tilted towards the head-side. In subsequent experiments (reported by Mosso's daughter in 1935), Mosso continued to investigate the effect of cognitive tasks on blood flow alterations with 'escalating' experimental paradigms that ranged from a 'resting' state to an 'active' cognitive state (Mosso, 1935). After the resting period, Mosso sequentially exposed the subjects to a wide range of stimuli of increasing cognitive complexity, such as a page from a news- paper, from a novel, from a manual of mathematics or philosophy, or a page written in abstruse language (Mosso, 1935). He reported that the increasing complexity of the stimulus modulated cerebral blood activity: the balance tilted faster towards the head side when the subject was reading a page written in abstruse language or belonging to a manual than it did when the subject was reading a newspaper or a novel (Mosso, 1935). Mosso stated that the increase in cerebral blood flow was thus proportional to the complexity of the cognitive task (Mosso, 1935), and he further measured the cerebral response to emotional stimuli, both in iso- lation and in interaction with cognition. In two other experiments, when Mosso's brother read a letter written by his spouse and when the student read a letter from an upset creditor, 'the balance fell all at once' (Mosso, 1935). To his surprise, Mosso noticed that subjects did not react equally to the same stimulus, and that this variability might have been due to differences in 'age…and education' (Mosso, 1935).

Temporal dynamics of cerebral activity

Mosso was always quite elusive in his interpretation of the exact temporal dynamics between the experimental stimuli and the modification of blood circulation. In a book published in 1883, he wrote that he had measured this temporal relationship exactly but would deliberately not provide further details as '…this is not the place to give numbers' (Mosso, 1883); his manner here is reminiscent of the famous demonstration omitted by Pierre de Fermat, on account of space constraints, and reported as a note scribbled in the margin of his copy of the ancient Greek text Arithmetica (Singh, 2012). Subsequently, in one of the last sentences of the work he published in 1884, Mosso noted that further details about the temporal dynamics of this relationship would be the object of 'a future Memoria [proceedings]' by Dr (Giulio) Fano, one of his assistants on that topic (Mosso, 1884). However, a search for this Memoria in the Archives of the Accademia dei Lincei revealed no publications written by Fano concerning the 'human circulation balance'. Moreover, it is rather surprising that during an important lecture, when the audi- ence included the Italian Royal Family, Fano never quoted the balance (Fano, 1910). Although the truth about these writings remains to be ascertained, we speculate that Mosso probably did not have access to the data obtained by Fano, or perhaps that Fano's reports were not considered original enough to be published by the Accademia dei Lincei. Mosso was probably not aware of the psychophysical investigations on reaction times undertaken by Franciscus Cornelis Donders (1868, 1969; but see also Luce, 1986), but his interest in temporal dynamics might have influenced his decision to bring Federico Kiesow to Turin; Kiesow had worked in the Wundt laboratory in Leipzig and was trained in the use of reacting time methods (Appendix 1).

Discussion and outlines

Angelo Mosso's initial claim was that local brain blood flow is intimately related to brain function (Raichle, 1998), and current researchers can recognize that the 'human circulation balance' can be considered as the conceptual basis of today's non-invasive functional neuroimaging techniques (Sandrone and Bacigaluppi, 2012). To our knowledge, the present paper is the first attempt to retrace Mosso's investigations with the balance, and specifically to discuss the operating mechanism in detail, as well as the studies performed, the experimental procedures and confounding variables, limitations and related crucial issues. Mosso wrote that the 'human circulation balance' allowed him to observe the same 'psychic fact' as that observable with the plethysmograph (Mosso, 1884). Nonetheless, we have no direct evidence that the balance was really able, as stated, to measure changes in cerebral blood flow during acts of cognition. Moreover, although it is still in existence, and despite its proven ability to measure blood volume changes in various organs (e.g. lungs, feet and hands), Mosso's original balance (Fig. 8) can no longer be used for experimental purposes. Accordingly, we cannot prove directly that it was actually capable of measuring alterations of cerebral blood flow during emotional and cognitive tasks.

However, the balance certainly fired popular imagination, and on 1 December 1908, a French newspaper reported that numerous people 'were passionate about the experiments of Professor Angelo Mosso' and enthusiastically believed that this device 'would soon fully explain the physiology of the human brain' and lead to new treatments for neurological and mental illnesses. Interestingly, Mosso was able to build his balance because of a unique combination of abilities and skills that ranged from his knowledge of medicine and physiology to the carpentering skills he learned from his father (Sandrone et al., 2012); later, collaboration with his mechanical assistant, Corino, additionally taught him how to build a machine piece by

piece (Mosso, 1935; Foà, 1957). Mosso's daughter remembers that when she was a child, her father used to nickname his balance as the 'metal cradle', the 'bed-balance', the 'machine to weigh the soul', or, more generally, 'one of my sisters', a conventional term he used for all his inventions, and who was always looking for 'a little window to look inside the human brain' (Mosso, 1935). In 1936, the scientist M.F. Lowe built a copy and a modified version of Mosso's balance in the Psychological Department at King's College London in order to repeat Mosso's experiments (Fig. 9). Unfortunately, due to some differences in the technique and in the experimental paradigms used, the two series of experiments are not strictly comparable (Lowe, 1936). Moreover, the exact relationship between increases in cerebral blood flow and cognitive activity still labours from knowledge gaps (Fox, 2012), such as the extent to which layer-specific neural processes are reflected in the functional MRI signal (Bandettini, 2012a; Goense et al., 2012). While cerebral blood flow during cognitive tasks, as detected by functional MRI, is

Figure 8 Photography of the balance (left) and the pneumatic sphygmograph (right) as they were found in the Scientific and Technological Archives, University of Torino (courtesy of Marco R. Galloni).
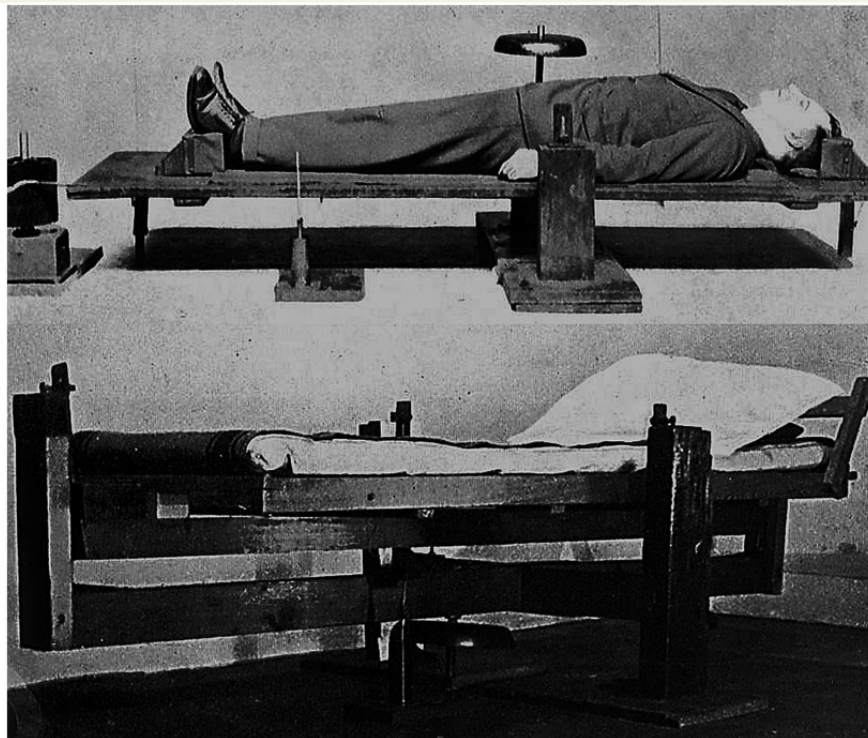

Figure 9 Mosso's balance (top) and its modified version (bottom) built by Lowe at King's College London (adapted and reprinted with permission from Lowe, 1936).

believed to exceed that of the resting state by 20–30% (Mildner et al., 2005), there is still no ultimate evidence that increases in blood flow are linked to a detectable increase in brain weight, nor are there any conclusive results concerning the relationship between global and regional blood flow variations and cerebral blood volume (Krieger et al., 2012). It is intriguing, and greatly to Mosso's credit, that work he published more than a century ago already contains many of the major themes and difficulties that characterize today's functional neuroimaging techniques (Bandettini, 2012b; Gazzaniga, 2009; Kandel et al., 2012). In this respect, the first point to note is that Mosso did not shy away from recognizing and discussing the low signal-to-noise ratio of his indirect study of brain function (Appendix 1), which is perhaps one of the most central issues in modern functional neuroimaging (Turner et al., 1998; Logothetis, 2008). In anticipation of what is frequently practised today, Mosso's balance included tools that detected and measured both head motion and breathing-induced oscillation, two of the most prominent sources of noise in functional MRI time-series. Mosso's use of a balance in conjunction with several other instruments (pneumograph, kymo-

graph and hydraulic plethysmograph, Fig. 5A–D), is also a good example of the current perception that a multimodal approach is required to increase the precision and resolution in the recording of physiological variables or to simultaneously record and stimulate the brain (Landini, 2009; Peruzzotti-Jametti et al., 2013; Peters et al., 2013). These tools for measuring head motion and breathing-induced oscillations very much anticipate the current rhetoric of 'physiological artefact removal', and resemble the current use of respiratory, electrocardiographical and other physiological measurements as a basis for confounding regressors in functional MRI (Lund et al., 2006; Iacovella et al., 2011; Birn, 2012). Mosso's prescience also comprehended the (still very current) tension between the need for substantial recording apparata, with which to derive ever more precise measurements, and an ecological set-up (Maguire, 2012). Mosso also considered the importance of psychological and demographic variables, and stressed both the importance of patient comfort in reducing unwanted artefacts (Russel et al., 1986; Byars et al., 2002), and the impact of variables such as age and education on experimental observations, variables that are often included today as covariates in data analysis. One of the most remarkably modern aspects of Mosso's work, however, relates to his choice of experimental designs (Mosso, 1935), which featured a comparison baseline or 'resting' period in an apparent block design (Petersen and Dubis, 2011; Sandrone, 2012) and in a parametric manipulation (Braver et al., 1997) to assess the cerebral response to increasingly complex acts of cognition (Dolan, 2008; Price, 2012). Interestingly, all the conditions were matched in their basic verbal nature and reading requirement, while differing in complexity. The increasing complexity of the stimulus in modulating cerebral activity recalls both the early approach to experimental design in the seminal PET word processing studies (Petersen et al., 1988, 1990; Posner et al., 1988), and the inception of cognitive subtraction in brain mapping, which is conceptually based on Donders' 19th century work on reaction time and thus extended from the temporal to the spatial domain (Donders, 1868, 1969; Posner, 1978; Luce, 1986). Finally, it is also noteworthy that Mosso's experimental team consisted of a medical student from his own institution and his own laboratory technician: the implicit, underlying sampling bias problem, is still very widely discussed in both psychological (Henrich et al., 2010) and brain research (Seixas and Basto, 2009; Chiao and Cheon, 2010). Mosso's balance inspired popular imagination to voice, through the writings of contemporary journalists, high enthusiasm for the invention that promised ultimately to 'fully explain the physiology of human brain' and to 'be used to treat neurological and mental illness': once again, these are sentiments and words that resonate in contemporary neuroimaging. In conclusion, paraphrasing the Nobel Laureate Jean Baptiste Perrin, weighing what was still invisible, Angelo Mosso started to increase our understanding of the visible (Perrin, 1926/1965). As the modern tools and techniques of functional neuroimaging continue to chart the road towards a greater understanding of the human brain, our rediscovery of Angelo Mosso's work allows us to firmly anchor the beginnings of several features of today's neuroscientific work in the 'human circulation balance'.

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Appendix 1

English translation of Mosso's speech to the Accademia dei Lincei entitled 'Applicazione della bilancia allo studio della circolazione sanguigna dell'uomo'. Atti della R Acad Lincei, Mem Cl Sci Fis Mat Nat 1884; XIX: 531-543

Application of the balance to the study of blood circulation in men

Memoria of the member Angelo Mosso delivered in the presence of the President, Academic Year 1883–84

Instrumental Part

The desire to simplify the tools that are used for studying blood circulation in men gave me the idea of placing an individual on the yoke of a balance, as shown in Figure 1 [Fig. 3 for Brain readers]. A wooden plank D, C can be made to oscillate about its centre when placed upon a steel fulcrum E, in the shape of a triangle, which rests one of its corners on a platform likewise made of steel. This section, which represents the yoke of a balance, is supported by a table A, B within which there are three openings: one in the middle and two at its extremities. A metre-long iron rod G, H, which has a large cylindrical cast iron weight at its lower end, is inserted into the openings; two additional rods, which meet at an angle, M, H, L, maintain the central rod in its position. The weight I moves along the rod thanks to a screw thread; a manual twisting motion enables the weight to move up and down the thread and thus to make the balance more or less sensitive. When a man is placed supine on the plank C, D, it is as if this were filled with water; or rather the man can be compared to a long bowl filled with liquid, which displaces at each movement of the plane upon which it is resting. It is enough to tilt the balance towards the head or towards the feet, by a few millimetres, or a centimetre at most, for blood to accumulate at one end in sufficient quantity to incline the balance to one side, which in turn requires a weight on the opposite side to return the balance to the horizontal position. This is one of the simplest and most conclusive experiments to demonstrate the great ease with which blood vessels dilate at the smallest change in pressure. If the balance is made so sensitive that, when it is empty, 100 grams placed at one extremity is sufficient to induce a tilt of approximately one centimetre, and a man is subsequently placed on the table C, D, with the balance reaching equilibrium, it will be seen that the balance does not move, regardless of the side towards which it tilts. This equilibrium is due to the accumulation of blood towards the head or the feet, even for depressions of less than a centimetre. To avoid this occurrence in the experiments that I will describe later, the weight I had to be placed lower; thus, by moving in the opposite direction to that of the blood, and given the length of the rod G H, the weight would act as a counterweight and brought the balance to equilibrium. To prevent the balance's oscillations from being too large, I placed two pieces of wood or elastic rubber upon the table A, B, acting as stops, these latter reduced the oscillations to a centimetre or less. To ensure that it was truly the shifting of blood towards the feet or the head that made the balance tilt, I concurrently recorded the volume changes in organs under similar circumstances. To obtain the recordings of the pedal pulse, I employed a sphygmograph that I had been using for several years [Footnote 1: It was with this apparatus that Dr Fano conducted some experiments in my lab on reflexive reactions in blood vessels, the results of which I reported to the Accademia dei Lincei in 1881]. It is an extremely simple instrument, which I had used in my application of the same methodology to the hand and foot, and it gave me very satisfactory results in the study of brain circulation. The methodology consists of transmitting the organ's volume changes to an ordinary tympanum and lever device; for the foot I made a half-boot of gutta-percha, which I closed with glassworker putty, as shown in Figure 2 [Fig. 4A for Brain readers]. Anyone can build this half-boot without difficulty or assi- stance. We wrap a piece of paper around the foot of the subject we want to study, and thus create a tailored half-boot paper cornet; using this cornet as a model, we then cut a sheet of gutta-percha, soften it in hot water and apply it to the foot, which has previously been well lubricated with oil or grease. The gutta-percha sheet is then joined at the sides and at the tip before being left to harden in cold water. These half-boots have to fit comfortably, so that the skin is not compressed and a small pocket of air remains between the foot and the boot; a cork opener can be used to make a small opening at the extremity of the boot, where a glass tube is inserted. This is the simplest and most practical sphygmograph to study the pedal pulse. To seal the half- boot, I usually use glassworker's putty. Thinner forms of this putty are preferable as they can be preserved in water and, when hardening is excessive, re-mixed by the addition of some oil drops until the putty becomes soft and sticky again. After the half-boot has been fitted to the foot, the putty is used to shape a border around the boot, the skin having been lightly greased to ensure better adherence with the putty. To study the pulse of the hand I often employ a gutta-percha glove, or simply a glass bottle from which I have cut the bottom, as shown in Figure 3 [Fig. 4B for Brain readers]. Here again, I employ the glassworker's putty for sealing purposes. This figure shows the drawing of the tympanum and lever I use to record the pulse in the more delicate experiments; the apparatus is much smaller than Marey's, although an ordinary tympanum may work just as well. In the experiments reported in this Memoria and in those that follow, since I was unable to use my water plethysmograph, I had to build a different plethysmograph, which works simply by air movements, and is much easier to handle. The device is shown in Figure 4 [Fig. 4C for Brain readers]. The outflow air from the half-boot, or the glass cylinder within which the hand or forearm is enclosed, enters from the bottom of a vase through tube F, and ascends vertically to a point above the level N M. An extremely thin metal bell A is kept in equilibrium on pulley C by counter-weight B, in which the inserted pen writes on the cylinder. Although this feature is not entirely necessary, the pulley's

hinges C turn upon two small wheels, so as to render the apparatus more sensitive. Vase D should be filled with petroleum essence, ether, or a liquid with little density, up to level N M. As can be seen in the figure, this apparatus is akin to a small gasometer; for this reason I named it a gasometric plethysmograph. Making the bell float by keeping it in equilibrium in a liquid, so that the volume of the gasses accumulated under it can be measured, is a task that presents several difficulties [Footnote: Note. For a plethysmograph to be useful as a measur- ing instrument it must abide by two conditions: firstly, it must accurately transcribe the volume changes of the organ whose circulation is under investigation; secondly, the surface pressure of the organ must remain constant. Several physiologists who perform plethysmographic research have built devices that differ from the liquid-movement plethysmograph proposed. I have never written a critique of these instruments because they lack the required conditions for an exact recording and for constant pressure, and they accordingly achieve much lower accuracy than that of my plethysmograph]. Everybody knows how this issue was solved with a spiral pulley in Hutchinson's spirometer. Nonetheless, I did not choose this compensatory method because it is not practical and also because the use of an asymmetric pulley introduces errors that are difficult to correct for. I preferred a partial compensation, and accordingly resorted to the use of extremely thin silver bells that move when immersed in a light liquid, thus producing negligible amounts of pressure. The bells are 20 cm tall and have a 30 cubic centimetre (cc) capacity. At their bottom end, there are two hooks to which the two silk threads that go to the pulley and hold the counterbalance are attached. The control experiments performed with this plethysmograph demonstrated that the additional pressure required lifting the entire cylinder above level N M, or the negative pressure resulting from entire immersion of the cylinder produced a maximum error of approximately 1 mm of water. This apparatus is so sensitive that when the rubber tube is filled with ether vapours, a minimal lifting of the tube is sufficient to let the vapours pass under the bell and lift it.

Influence of respiration-related movements on blood circulation

If the gravity centre of the balance is shifted to very low, so as to confer the necessary sensitivity that prevents the balance from inclining too easily, when an individual is placed on and in equilibrium, the balance will continuously oscillate, as dictated by the respiratory rhythm. During inspiration, the balance tilts towards the feet. This movement, however, is not exactly synchronous with the respiratory movements, but rather is slightly delayed as a result of the inertia of the balance itself and of further factors that we will discuss later. Figure 6 [Fig. 5D for Brain readers] depicts the traces of an experiment in which I recorded respiratory movements with Marey'spneumograph and a pen that had been attached to the extremity R of the balance, as shown in Figure 1 [Fig. 3 for Brain readers], to trace line B. From the trace it is clear that the movement of the balance B matches the respiratory rhythm R with a short delay. PP indicates the correspondence of the two pens. Because it would be legitimate to ask whether these oscillations depend on the movement of the intestinal masses induced by diaphragmatic contraction, I fixed a support, like the back of a high chair, to the balance, so that the subject would be in a sitting position and the diaphragmatic motions would take place vertically on the fulcrum of the balance; nonetheless, as seen in Figure 6 [Fig. 5D for Brain readers], the respiratory oscillations are still evident. Line B is very different from the line in Figure 5 [Fig. 5A for Brain readers], because, in this case, the balance tilts and hits an elastic rubber cork, thus producing a greater number of oscillations. All things considered, it is easy to recognize that this increase derives from a real redistribution of blood to the extremities at each inspiration, when the feet swell and the hands diminish in volume. Figure 7 [Fig. 5B for Brain readers] simultaneously records the respiration with Marey's pneumograph placed around the thorax (line R), the foot pulse with the air sphygmometer (line P), and the hand pulse with the same method (line M). The oscillations of the balance, recorded on the foot-side, are shown in line B. What emerges from these traces is an antagonistic relationship between the respiration-induced volume changes in the hand and the feet. I would like to first point out to the reader that with the balance it is possible to recognize and record spontaneous movements of the blood vessels that I had already studied in humans with a plethysmograph and named undulations. For as yet unknown reasons, constrictions and dilations of blood vessels at the extremities produce, in humans, a movement of the blood that makes the balance incline to one side or the other. Figure 7 [Fig. 5B for Brain readers] depicts one of these undulations. The left-hand side of the previous section of this tracing (not shown here) recorded a dilation of the blood vessels of the foot, the reasons for which elude me completely. The volume of the extremities noticeably increased throughout six respiratory movements, and the balance inclined downward and stabilized in this position. This state persisted for three respiratory movements, which were marked by a progressive contraction of the foot's blood vessels, as shown by the downwards trend of line P. Line B shows that, after the decrease in foot volume, the balance resumed its oscillatory motion. These undulations, which are produced during sleep and restful wake for internal causes, are unknown to us and must be distinguished from other, psychically-induced types of blood movement, which we will discuss later in this Memoria. A close examination of the traces of the hand and foot shows that they have an antagonistic relationship. Indeed, in point A, towards the end of the inspiration, I found that the volume of the hand diminished, whereas that of the foot increased. In my previous work on brain blood circulation, the numerous experiments assessed the influence of respiratory movements on blood pressure; diversely from the physiologists who preceded me, I stressed the importance of the volume change at the extremities, and specifically that said changes derived

from to thoracic inspiration and abdominal pressure [Footnote: A. Mosso. Cerebral blood circulation in man. Memorie of the Reale Accademia dei Lincei, 1880, Vol. V, p. 237]. Without wishing to review this controversial issue yet again, my observations argue that abdominal pressure, which increases during inspiration, impedes blood as it returns towards the heart, thereby producing an engorgement of venous blood in the legs. In other words, we see in the lower part of the body what typically happens when an obstacle hinders a river's flow: a slow surge takes place on the spring side of the river. To analyse the speed at which this venous blood backflow occurs, and to distinguish it from a greater arterial blood afflux, I simultaneously recorded the time at which the veins of the foot and the veins between the knee and the hip engorged. To this end, I built a gutta-percha boot made of two matching parts that were hermetically sealed with putty so that an air drum could measure the pulse and volume changes along the whole leg. On the other leg I attached the half-boot described above to the front of the foot. Figure 8 [Fig. 5C for Brain readers] demonstrates that the volume of the whole leg does indeed increase faster than that of the foot during inspiration; in the foot, the engorgement appears with a lag of approximately 2 seconds. I find it difficult to conceive any explanation of this result other than as a venous engorgement; from now on, in order to assess the influence of respiration on venous circulation when studying volume changes in the brain, hands and feet, it will be necessary to take into account the lag linked to this venous blood reflux. When respiration-related volume changes in the brain and foot appear to match, it is important to consider that the inspiration-induced volume increase in the foot might take place so late that it can occur simultaneously with the volume increase seen in the brain during the subsequent inspiration; conversely, the cerebral volume decrease during inspiration can occur simultaneously with the inspiration-related volume decrease in the foot. I will discuss these results in a future Memoria on the topic of cerebral blood circulation in man. The complete opposition that exists between the venous circulation superior and inferior to the diaphragm is even clearer when the respiratory movements are exaggerated. In Fig 1 of table I [not shown], I recorded the respiratory motion of the thorax, line T, the abdomen, line A, and the pule at the foot P, and at the hand M; as soon as inspiration begins it can be seen that the volume of the foot increases, while that of the hand decreases. The antagonism between these two changes remains throughout the inspiratory effort; as soon as expiration starts, the leg veins can unclog and the veins of the hand swell and regain their volume. If one tries to take a deep breath with the diaphragm alone, and the thorax motionless, the volume increase in the legs is much greater, while the volume decrease in the hand is barely visible. Conversely, if the thorax is greatly dilated and the diaphragm is not fully contracted, the inspiratory stagnation in the inferior extremities' veins can disappear because of the absence of venous pres- sure in the abdominal cavity. When a person lies on the balance, it takes quite some time before the foot's vessels unclog and the blood which had accumulated, because of gravity, in the inferior extremities uniformly distributes to all organs. To avoid any discomfort to the experimental participants when they had to remain still for a long time, I padded the case D C [Fig. 3], and made markings on the borders of the case in order to notice any involuntary movement of the hands. If the weights placed in R allow equilibrium to be achieved soon after a person assumes a supine position, the legs become rapidly lighter; to keep the balance horizontal it is necessary to continuously add weights. To measure the quantity of blood that flows from the foot towards the middle of the body I place a glass by the feet and fill it, from a 1/10 cc calibrated burette, with as much water as is needed (minute by minute) to keep the balance in equilibrium. The amount of blood that flows away from the inferior extremities when someone moves from a vertical to a horizontal position is greater than is commonly believed. The eye is unable to detect these changes even though, for the two feet together, the changes invariably exceed 100 cc. When the ambient temperature is high, the changes are much greater. Once, when a subject kept both feet in hot water for 10 minutes before participating in the experiment, the difference reached 260 cc after half an hour. I will cover this phenomenon in a future Memoria, in which I will relate my research on the tonicity of blood vessels in humans; however, I would like to point out that the balance here described allows cer- tain features of human blood circulation to be studied much more easily than does the plethysmograph; for instance, the effects of warm and cold temperature and humidity on blood vessels. When the plethysmograph is sealed, it is impossible to ensure that blood vessels do not get compressed. Although I have yet to deal with the issue experimentally, I believe that use of the balance might enable the diagnosis of serum draining in the abdominal cavity, a diagnosis that cannot be determined by any other means.

Determining the amount of blood that accumulates in the lungs during respiratory motions

In my first work on this topic [Footnote: Sulla circolazione del sangue nel cervello dell'uomo. R. Accademia dei Lincei Vol. V 1880, Chapter X, XI; and Uber den Kreislauf des Butes in menschlichen Gehirn. Leipzig 1881] I built a device which measured the amount of blood that accumulated in the lungs at each respiratory motion. Despite the fact that those recordings were performed outside the thoracic cavity and by the means of artificial circulation, the experimental set-up was so close to normal conditions that I felt it left little doubt concerning the amount of blood that accumulates over a certain period of time in the lungs during deep inspiration. Using the balance I confirmed in humans the results I had obtained with artificial circulation in explanted organs. Indeed, I observed that if one makes a deep inspiration when the balance is in equilibrium it tilts first towards the feet and then, as soon as the inspiration finishes, it inclines towards the

head, where it momentarily rests. For an approximate measure of the amount of blood that is accumulated in the thorax, I thought it would suffice to place a subject in equilibrium on the balance, have him repeatedly take deep breaths and then determine the weight that had to be removed from the thoracic area to re-establish equilibrium. Because said removal of weights presented practical difficulties, I devised a system whereby a half-litre pitcher with an opening at the bottom was positioned by the thorax; a drain in the form of a rubber tube extended from the bottom of the pitcher, and bent at 90 degrees to the balance's fulcrum, point E. Having filled the pitcher with water, and with the subject in equilibrium, I would ask him to perform one or two inspirations.

I would then open the tube's faucet so as to drain off sufficient water for the balance not to remain tilted at the head end. This approach circumvented the problem of having to touch the balance to re-establish equilibrium and thus of generating undulations. The drained water was then collected in a cylinder and graded in cc, allowing the approximate measurement of how much liquid had moved towards the lungs. As of the very first experiments, I noticed that when people made a series of deep inspirations a few minutes after laying down on the balance, this latter seldom reverts to equilibrium, even after a lengthy period of time. The reason for this has to do with something that resembles inertia, an imperfect elasticity, which I would describe as a state of blood vessel mellowness. The fact is that when vessels are filled (and hence dilate) excessively, irrespectively of the cause they never revert completely to the same state. When one is in a vertical position, the leg's blood vessels dilate and engorge slowly because of gravity; if one then lies supine, the vessels do not unclog completely; the presence of a residual amount of blood would lead one to believe that the blood vessels have remained inert. Indeed, when there is a diminution in the blood vessels' content on account of neural or mechanical causes, these vessels do not retain their initial volume because of their elastic properties: the dilatation force exercised by the heart and blood pressure is diminished. We thus have to assume that the same is true of the lungs in a living animal. To avoid potential errors potentially deriving from the un-clogging of blood vessels in the leg, when I performed experiments on respiration, I ensured that the participant initially spent at least one hour supine on the balance. I will now report on a set of experiments I performed on the 15th of February.

1st experiment

Giorgio M., a worker in my own laboratory, is a burly 25 year-old man, 1.62 m tall, 61.5 kg in weight, and has a pulmonary capacity of 3500 cc. At 2:15 he lay on the balance and took a nap. After an hour the legs appeared to be entirely un-clogged, since the balance was mostly in equilibrium and oscillating regularly in keeping with the respiratory rhythm. At 3:25 he took two deep breaths. Immediately the balance inclined towards the head. I then opened the jug's faucet to bring the balance back to equilibrium, and 130 cc was drained. The balance spent just a few seconds in a horizontal position and then, in keeping with the respiratory rhythm, exhibited a tendency to tip towards the feet. I thus had to add water to the jug on the side of the lungs. At 3:32 the balance resumed oscillating. However, 100 cc of water was still missing. I thus performed a double-check: I poured 100 cc of water into the jug by the thorax to return to the previous conditions, and then opened the faucet and noticed that once 105 cc had been drained, the balance resumed its oscillations. At 3:38 I asked for a series of deep breaths. I had to immediately remove 105 cc of water for the balance to tip towards the feet. However, after 1 minute the lungs were so un-clogged from the blood that had accumulated that I had to add water to the jug to keep the balance in equilibrium. At 3:40 I added another 65 cc to re-establish the previous oscillation. Five minutes later, 175 cc of blood was seen to have accumulated on the side of the head, since the same amount of water was missing from the jar by the thorax. Giorgio was resting. At 3:48 I asked him to perform a forced expiration, where upon the balance inclined towards the legs. I had to add water to the jug by the thorax. Two minutes later the balance was again in equilibrium: 125 cc was missing from the jug. I am confident that the subject did not move, so the 125 cc of blood likely accumulated in the lungs.

2nd experiment

The subject was V.G., a 22 year-old medical student, 1.80 m tall, 73 kg in weight and with a pulmonary capacity of 4000 cc: on the 11th of February I placed him in equilibrium on the balance; when it oscillated regularly in keeping with respiration, to assess the sensibility of the scale, I placed a weight of 20 g by the knees and observed the scale tilt towards the feet. At 4:10 I manually kept the balance still at the foot end, and I had Mr G. perform five deep inspirations. Once these were performed I released the balance, which tilted immediately towards the head. I then had to remove 360 cc of water from the jug so that the balance oscillated towards the feet; successively, as the lungs became un-engorged, I had to add water to keep the balance horizontal. At 4:14 there was still 220 cc remaining on the side of the lungs. The balance had a continuous tendency to tilt towards the feet, so I accordingly added water on the side of the lungs. At 4:23, without anything changing or any other external cause, the balance tilted towards the head; I was thus forced to drain more water to re-establish equilibrium. A total of 420 cc was drained before the balance returned to equilibrium. Prompted by this unusual phenomenon, I asked Mr G. how he was feeling; he answered that after the apnea he had

experienced some vertigo, and that now, without knowing why, he felt that the blood was coming back to his head. I have observed this phenomenon in several other subjects. There is an accumulation of blood on the side of the lungs because of the apnea. Subsequently, the blood has a tendency to return to the previous state of equilibrium and returns to the peripheral parts of the body, following which there is a second movement of blood towards the core of the body, for reasons that I cannot explain.

The movement of human blood vessels as studied with the balance

All of the phenomena concerning blood circulation that I observed in humans with the plethysmograph are equally observable with the balance. Indeed, they appear even more clearly because the apparatus is simpler and thus the expression of the phenomenon is more sensitive. I report a trace to demonstrate the method I developed in these observations. Generally, I recorded several traces simultaenously: respiration, the pulse of the hand and foot, and the movements of the balance. On 21st April 1882, I ask my lab worker Giorgio M. to drink a little bit of wine at lunch, because I wanted to perform an experiment on his pulse. At 2 we began: he lay on the balance, while I attached the gutta-percha half-boot to the right foot and the gutta-percha glove to the right hand. The left arm was resting with the elbow on the edge of the table, and the forearm was on the chest, so that the hand remained at the level of the sternum. The left arm was lifted and rested on a pillow behind the head so as to gently wrap around the occipital. I fitted Marey's pneumograph around the chest. I took great care that the plastic tubes that ran to the drums were all of the same length and did not impede the movements of the balance. The pulse was just as strong in the hand as it was in the foot. Giorgio napped lightly. Every time I talked to him, I noted a strong contraction of the blood vessels in the hand and foot, and the balance inclined towards the head. At the beginning, the extremities' blood vessels were highly irregular, and exhibited pletysmographic undulations that were so strong that the curves of the hand and the foot's pulse would sometimes not correspond and appear entirely independent. Whenever Giorgio fell asleep, the balance tended to tilt and rest towards the foot end. Any external noise produced a contraction of the extremities' blood vessels and a consequent inclination of the balance towards the head. This phenomenon is very clear in the recordings in Figure 2, Table I [not shown], where a noise modified the respiration and the circulation, but did not wake Giorgio up. At 3, the balance was oscillating regularly in keeping with the respiratory rhythm, line B. At point R, I made a noise using my hand to hit the knob of an electric key, just like those used to transmit telegrams. After mark R, we can see that, in line I, several seconds passed before any sign of contraction in the hand's vessels was noticeable, and it took a few seconds more for the contraction to be noticeable in the foot. I cannot make further considerations concerning the time that elapses between the moment when a psychic impression is made and the moment when a reflexive response is observed in the blood vessels, since this was the subject of a study performed in my laboratory by Dr Fano, which will be reported in a future Memoria as an integration to the preliminary communication made to the Accademia dei Lincei in 1882. Similarly, I cannot further comment on the time elapsing between a psychic event, or any type of excitation, and a change in respiration, since this too will be included in a future Memoria. Comparing the thoracic respiratory trace T with line I, which marks the time when the noise was made, we see that the thorax stopped almost immediately at the beginning of the inspiration. When the contraction of blood vessels in the hand and foot reached its maximum, the balance inclined towards the head, and rested there throughout the time in which the foot's volume was decreased. The line of the foot's pulse is incomplete; the horizontal section is produced by a pen which is held by the drum below it. After rapid contraction, during which the subject did not wake up, the blood vessels of the hand and foot relaxed and followed the curve that can be observed (complete) in the hand's recording. In comparison with the respiration, we can see that, immediately after a brief stop, some faster and deeper respirations followed, before reverting to the previous rhythm. The balance's trace, line B, shows some sinu- osity that corresponded to the pulse's rhythm. I could show other traces where the cardiac pulsation traces are more evident, but it would not be very helpful since the inertia of the apparatus only allows the pulse's frequency to be recognized. In conclusion, the above shows that all of the phenomena I previously observed with the plethysmograph concerning blood vessels in humans, including pulsations, respiratory oscillations, spontaneous movements of blood vessels and the undulations that correspond to psychic facts, are equally visible with the balance.